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Rapid Technique for Estimating DNA Binding Used to Evaluate 1-Nitropyrene Adduct Formation

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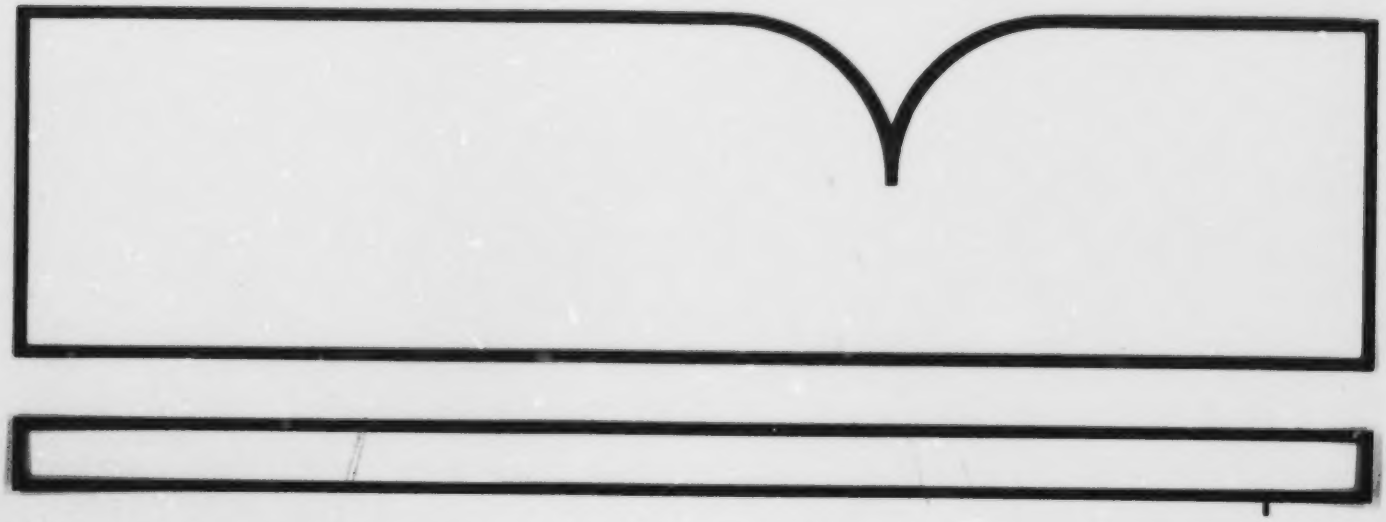

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A RAPID TECHNIQUE FOR ESTIMATING DNA BINDING, USED TO EVALUATE 1-NITROPYRENE ADDUCT FORMATION

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ABSTRACT

A simple and rapid procedure for estimating binding of radiolabelled material to DNA and protein is described. Protein was extracted from lysed rabbit alveolar macrophages with chloroform: iso-amyl-alcohol:phenol extraction. Nucleic acids were precipitated from the lysate, and hydrolysed with protease and NaOH to remove residual protein and RNA respectively. Bound radioactivity was quantitated by precipitation of DNA onto glass fiber filters. Protein labelled with ^{3}H-leucine and DNA and RNA adducts formed from 1-nitro[^{14}C]pyrene by xanthine oxidase were used to define this procedure. ^{14}C was shown to be bound to endogenous protein and DNA isolated from rabbit alveolar macrophages that had been incubated with 1-nitro[^{14}C]pyrene.

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INTRODUCTION

Growing awareness of the health hazards arising from environmental pollutants, particularly polycyclic aromatic hydrocarbons (PAH) has fostered interest in the use of short-term bioassays for rapid identification and characterisation of potentially hazardous complex mixtures and of individual compounds[1]. The relationship between mutagenicity and carcinogenicity has been well documented[2], and since nitro-PAHs are among the most potent bacterial mutagens detected in the Ames assay[3], their presence in combustion emissions[4,5] may contribute substantially to the genotoxic burden of ambient air. Many current investigations are now directed towards evaluating whether nitro-PAH are as active in mammalian systems as in bacteria, since their potency in microbial assays is thought to depend essentially on the presence of nitroreductase enzymes in the bacterial tester strains used[6].

In addition to the numerous mammalian mutagenicity assays already in use, we sought a more direct means of evaluating gene tic damage which could be used in freshly-isolated cells and tissues, and eventually in organs from in vivo exposures. DNA binding appears to correlate well with mutagenicity and carcinogenicity for a number of PAH[7-9], and techniques have been documented for obtaining DNA of high purity, covalently bound to radio-labelled substrates[10-15]. While these techniques are invaluable in purification of DNA for detailed structural studies, they require the use of laborious steps and dedicated equipment (hydroxylapatite column chromatography, cesium chloride or sucrose gradient centrifugation) and limit the number of specimens which can be assayed simultaneously.

The procedure which we describe here is much more rapid, requires no expensive equipment not commonly available in the biochemistry laboratory, and is suited for use as a routine analytical tool. We have developed this process to measure adduct formation between cellular macromolecules and radio-labelled nitro-PAH,

and report here its use and validation in characterizing the nucleic acid binding of 1-nitro[^{14}C]pyrene in some mammalian *in vitro* systems.

MATERIALS AND METHODS

Chemicals:

1-Nitro[^{14}C]pyrene (^{14}C-NP; 10μCi/μmole, radiochemical purity better than 99.7%) was synthesised by Midwest Research Laboratories, Kansas City, MO., ^{3}H-leucine (57 mCi/mMole) was purchased from Amersham Corporation, Arlington Heights, IL. and Aquasol II from New England Nuclear, Boston, MA. DNA (Type I, from calf thymus), RNA (Type XI, from bakers' yeast), xanthine oxidase (Grade I, from buttermilk) and RNase (Type IA, from bovine pancreas) were purchased from Sigma Chemical Co, St Louis, MO. Proteinase K was obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN. All tissue culture media and antibiotics were purchased from GIBCO Laboratories, Grand Island, NY. Fetal calf serum was obtained from Sterile Systems Inc., Logan, UT. Chloroform (spectro grade) was purchased from Burdick and Jackson, Muskegon, MI; isoamyl alcohol from Fisher Scientific, Raleigh, NC; and phenol (99+%) from Aldrich Chemical Co., Milwaukee, WI. All other materials were purchased from reputable suppliers at the highest grade of purity available.

Animals and Cell Preparation:

Rabbit pulmonary alveolar macrophages (RAM) were obtained from New Zealand White rabbits (Dutchland Laboratory, Denver, PA.) by the pulmonary lavage procedure described previously[16]. The cells were harvested by centrifugation then resuspended in M199 medium containing 10% fetal calf serum, penicillin (100μ/ml), streptomycin (100μg/ml) and Kanamycin (100μg/ml). This medium was used in all subsequent incubations. The viability of the RAM was verified by

exclusion of trypan blue, and in all cell preparations used was better than 90%.

Incubation of RAM and Protein Labelling:

Cells were seeded in 150 cm^2 Corning culture flasks and incubated at 37°C under a humidified atmosphere of 5% CO_2 in air. Cellular protein was labelled by incubating RAM (20 x 10^6 cells/30 ml medium/flask; 5 flasks) with 0.25 μCi of ^{3}H-leucine/ml [17]. For the determination of cellular adduct formation, 15 x 10^6 cells/30 ml medium/flask (7 flasks) were incubated with ^{14}C-NP (2 μg/ml). After 20 h of exposure, the cells were removed by trypsinizing then pelleted by centrifugation. All procedures involving ^{14}C-NP were routinely carried out under yellow light.

Formation of Specific Nucleic Acid Adducts:

Calf thymus DNA (2 mg/ml) or yeast RNA (2 mg/ml) was incubated with ^{14}C-NP (20 μM) xanthine oxidase (0.5 u/ml) and hypoxanthine (0.5 mg/ml) under N_2 [18]. After 2 h the incubations (10 ml) were terminated by addition of an equal vol of chloroform: iso-amyl alcohol:liquefied phenol (24:1:25 by vol, CIP) and the adducts isolated and quantitated as described below.

Cell Lysis and Isolation of Nucleic Acid Fraction:

The cell pellets were resuspended in 10 ml of a lysing buffer composed of 50 mM Tris-HCl: 10 mM EDTA: 1% w/v sodium dodecyl sulphate: pH 8.0. The mixture was vortexed, then left to digest at room temperature, with additional vortexing every 10 min. After 30 min, an equal vol of CIP was added, and mixed well by vortexing every 5 min. The resulting emulsion was separated by centrifugation (3000g, 15 min, at 4°C) and the aqueous (upper) layer reextracted as before with CIP. The aqueous phase was recovered, and made 0.1 M with respect to NaCl. Two vol of cold EtOH were added; DNA precipitated almost immediately, but in experiments where RNA was isolated it was necessary to leave the mixture overnight at -20°C to obtain complete precipitation. The precipitated nucleic acid fraction was harvested by centrifugation (300g, 15 min, 4°C),

then the pellet was suspended in 0.15 M NaCl: 0.015 M sodium citrate buffer (pH 7.0, 1-2 ml). The DNA content of this solution was assayed by the diphenylamine colorimetric method[19], using calf thymus DNA as a standard. RNA was determined by the orcinol colorimetric method[20].

Isolation of Protein and Quantitation of Bound Activity:
The two CIP fractions remaining were combined and an equal volume of acetone was added to precipitate the protein contained in these layers. The protein was recovered by centrifugation (300xg, 15 min, 4°C) and washed three times each with acetone, ether and 95% ethanol to remove unbound radioactivity. After overnight desiccation, protein was dissolved in 1-2 ml 10M NaOH at 37°C. A small aliquot was then neutralized with concentrated HCl and aliquots were taken for scintillation counting and total protein determination[21].

Quantitation of ^{14}C Bound to Nucleic Acids:
Duplicate aliquots (100 µl) of the redissolved nucleic acid fraction were pipetted onto glass microfibre filters (Whatman LTD, Maidstone, Kent, UK). The filters were washed by swirling in cold 10% trichloracetic acid (TCA) for 30 min, then three times with cold 5% TCA for 5 min, then three times with 95% EtOH for 5 min. The filters were dried in air, dissolved in Aquasol-2 (10 ml), and the ^{14}C precipitated on them was determined by liquid scintillation counting (Packard Model 460C, Downer's Grove, IL), after chemiluminescence had decayed. The efficiency of counting was determined from a previously-established calibration curve, by means of an external standard. Specific binding was calculated from the specific activity of the ^{14}C-NP and the previously-determined DNA (or RNA) content.

Hydrolysis of Protein and RNA:
To remove residual protein, the DNA solution was incubated with Proteinase K (1 mg/ml) for 2 hr at 37°C, then an aliquot was counted again on glass microfibre filters. RNA was hydrolysed by

incubation in 0.4 M NaOH at 46°C for 1 hr[44], or by incubation with RNase, (0.1 mg/ml for 18 hours at 37°C) and the solution was counted again after precipitation onto the filters.

RESULTS

Radioactivity was associated with the acid-precipitable nucleic acid fraction following incubation of ^{14}C-NP and xanthine oxidase with either DNA or RNA (Table 1). Xanthine oxidase was therefore able to catalyse the binding of NP-derived ^{14}C to both DNA and RNA, at levels of 1-2 nmol/mg nucleic acid; these values are comparable to those reported for DNA[18]. These nucleic acid adducts purified by CIP extraction were taken both separately and together through the hydrolysis steps to eliminate, successively, protein and RNA (Table 1). Neither was affected by the protease treatment, indicating both that the protease used did not contain significant residual nuclease activity, and that protein carryover from the xanthine oxidase in the incubation was negligible. The DNA was minimally affected (less than 3% decrease in total precipitable ^{14}C) by the NaOH hydrolysis, but less than 5% of the RNA-associated radioactivity remained acid-precipitable following this treatment. RNase treatment was comparable to alkaline hydroxysis in that 97% of the ^{14}C-activity associated with RNA was no longer acid-precipitable after 18 hours of treatment (results not shown). However the time necessary to acheive a comparable result (18 hrs versus 1 hour) made the alkaline hydrolysis method more practical for routine use. When a mixture of ^{14}C-bound DNA (31%) and RNA was taken through the whole hydrolysis procedure, 40% of the original ^{14}C remained acid-precipitable (Table 1), indicating that where RNA adducts were present in large quantities, the majority of these will be removed by hydrolysis, but that up to 12% of residual RNA-bound radioactivity could be anticipated.

In order to verify the effectiveness of the method for separating labelled protein from DNA in whole cells, RAM cells were

TABLE I

Efficiency of Purification of ^{14}C-Labelled Nitropyrene-Nucleic Acid Adducts[a]

Sample	DNA[b]	RNA[b]	DNA+RNA[c]
As isolated	38,974±3351	41,811±2410	100
After Proteinase K Treatment	36,752±1353 (1.4)	45,250±1128 (0)	103±4
After Alkaline Hydrolysis 15 min	---	1572±357 (96.4)	---
30	42,796±10,753 (0)	1537±347 (96.5)	---
60	36,616±1521 (2.4)	1913±235 (95.6)	40±1
120	---	1622±401 (96.3)	---

[a] ^{14}C-NP was incubated with xanthine oxidase and either DNA or RNA, and the nucleic acid adducts formed were isolated as described in Materials and Methods.

[b] dpm/mg, determined as described in text (mean±S.E.M., n=3-9); the number in parentheses is the percentage of activity lost compared to the isolated fraction (mean of 3-9 determinations).

[c] 20770 dpm of ^{14}C-labelled DNA and 46230 dpm of ^{14}C-labelled RNA were mixed then treated as described. The values given are the total remaining acid-precipitable activity expressed as a percentage of the activity in the original mixture.

TABLE 2

Estimation of ^{3}H-Labelled Protein Associated with Nucleic Acids Isolated from Cells Exposed to ^{3}H-Leucine[a]

Sample	Protein Fraction		Nucleic Acid Fraction	
	Total Radioactivity[b]	Specific Activity[c]	Total Radioactivity[b]	Protein Content[d]
Lysate	924,383±138,581	92,755±10,191	---	---
After CIP Extraction	882,774±56,493	98,451±804	9447±3529 (1.0)	96±3
After Proteinase K	---	---	1606±599 (0.2)	17±6
After Alkaline Hydrolysis	---	---	318±126 (0.03)	3±1

[a] Rabbit alveolar macrophages (98.7±6.3x10^{6} cells) were incubated for 20 h with ^{3}H-leucine (35μCi; 57μCi/μmole). Protein and nucleic acids were isolated as described in Materials and Methods.

[b] Values represent total radioactivity in the recovered fraction, in dpm (mean±S.E.M, n=4-6). The number in parentheses is the percentage of protein label remaining in the nucleic acid fraction.

[c] Expressed as dpm/mg protein (mean±S.E.M., n=4).

[d] Expressed in μg; determined from specific activity measured in isolated protein fraction.

incubated with ^{3}H-leucine and nucleic acids were isolated by the same procedure. The CIP extraction removed more than 99% of the ^{3}H taken up by the cells (Table 2). Treatment with protease plus alkaline hydrolysis further reduced the total radioactive content of the nucleic acid fraction to 318 dpm, corresponding to 4 millionths of the radioactivity to which the cells were originally exposed, and 0.03% of the label taken up into the cells. The recovery of DNA from *ca.* 10^{8} cells was 350 µg (3.41±0.1pg/cell). Hofmann[23] reported 2.9±0.1pg/cell in non-activated rabbit pulmonary alveolar macrophages and 4.5±0.9pg/cell from activated macrophages.

When ^{14}C-NP was incubated with RAM, acid-precipitable radioactivity became associated with both the protein and the DNA fractions (Table 3). Based on the specific activity of the ^{14}C-bound protein, and the maximum extent of possible protein carryover calculated from Table 2, less than 20 dpm/mg of the apparent DNA binding would be spurious counts derived from protein contamination. Alkaline hydrolysis did not decrease the acid-precipitable radioactivity, indicating that virtually no ^{14}C was bound to RNA.

The total time taken to run the whole assay is two days, and the number of samples that can be handled simultaneously in one batch is limited essentially by the capacity of the centrifuge

TABLE 3

Estimation of ^{14}C Bound to Macromolecules from RAM Cells Exposed to ^{14}C-1-NP[a].

Sample Description	dpm/mg[b]	pmole/mg[c]
Isolated Protein	103±25	5±1
Nucleic Acids before Hydrolysis	159±44	7±2
Isolated DNA after Hydrolysis	230±88	10±4

[a]As described in text
[b]Mean ±S.E.M., n=3
[c]Mean ±S.E.M., n=3 based on specific activity of ^{14}C-1-NP

used to break the CIP emulsion. Approximately one hundred samples can reasonably be processed by one person in a two-week period.

DISCUSSION

We have developed a procedure for rapid routine determination of macromolecular adducts which we have used to evaluate the nucleic acid binding of NP. While no single step of this procedure is novel in itself, and the end product may not lend itself to analysis and identification of bound residues without further purification, our technique offers us a straight-forward, efficient and inexpensive means of assaying adduct formation. CIP extraction effectively removes the major portion of any labelled protein from the nucleic acid fraction; this is followed with hydrolysis to remove residual protein and RNA. Precipitation of the remaining DNA onto glass fibre filters, and extensive washing of these, reduces the possibility that unbound radioactivity may still be associated with the DNA. By using endogenously labelled protein and nucleic acid adducts formed *in vitro* with a purified enzyme system, we have shown that this technique is specific enough for routine analytical purposes, and expeditious so that approximately one hundred assays can be carried out over a two-week period.

The ability to recover measurable quantities of DNA from a relatively small number of cells is also of value, particularly when working with freshly-isolated cells from small organs where large populations are not available, or when DNA adducts are to be determined in organs from laboratory animals exposed to radioactive compounds *in vivo*. The latter type of experiment historically has required the use of massive doses of radioactivity (3 to 30 mCi)[24], which is often undesirable as well as simply unavailable.

We have demonstrated that NP forms DNA adducts in a whole cell preparation derived from mammalian sources, and *in vitro* incubations with xanthine oxidase. This cytosolic nitroreductase functions optimally under anaerobic conditions, and has been shown to

catalyse binding of ^{3}H-NP to DNA[18]. Our results indicate that RNA is as effective as DNA in trapping electrophilic intermediates which may be formed during the reduction of NP *in vitro*.

RAM, which posess drug metabolising capabilities[25], are located in the airways of respiratory tissue, where O_2 is plentifully supplied, and are unlikely to foster predominantly anaerobic enzyme activities. Therefore formation of macromolecular adducts from NP in macrophages may occur by other mechanisms. Studies are in progress to evaluate the disposition of ^{14}C-NP in these cells, and identify the metabolites formed (King et al, manuscript in preparation).

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REFERENCES

1. Huisingh, J., Bradow, R., Jungers, R. Claxton, L., Zweidinger, R., Tejada, S., Bumgarner, J., Duffield, F., Waters, M., Simmon, V., Hare, C., Rodriguez, C., and Snow, L. Application of bioassay to the characterization of diesel particle emissions. In: Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Plenum Press, New York, 1978, p. 383.

2. McCann, J., Choi, E., Yamasaki, E., and Ames, B. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Natl. Acad. Sci. U.S.A. 72, 5135, 1975.

3. Mermelstein, R., Kiriazides, D.K., Butler, M., McCoy, E.C., and Rosenkranz, H.S. The extraordinary mutagenicity of nitropyrenes in bacteria. Mutat. Res. 89, 187, 1981.

4. Schuetzle, D., Lee, F.S., Prater, T.J., and Tejada, S.B. The identification of polynuclear aromatic hydrocarbon derivatives in mutagenic fractions of diesel particulate extracts. Int. J. Environ. Anal. Chem. 9, 93, 1981.

5. Nishioka, M.G., Petersen, B.A., and Lewtas, J. Comparison of nitro-aromatic content and direct-acting mutagenicity of diesel emissions. In: Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry, Battelle Columbus Press, Columbus, Ohio, 1981, p. 603.

6. Rosenkranz, H.S., McCoy, E.C., Mermelstein, R., and Speck, W.T. A cautionary note on the use of nitroreductase-deficient strains of Salmonella typhimurium for the detection of nitroarenes as mutagens in complex mixtures including diesel exhausts. Mutat. Res., 91, 103, 1981.

7. DiGiovanni, J., Romson, J.R., Linville, D., Juchau, M.R., and Slaga, T.J. Covalent binding of polycyclic aromatic hydrocarbons to adenine correlates with tumorigenesis in mouse skin. Cancer Let., 7, 39, 1979.

8. Wigley, C.B., Newbold, R.F., Amos, J., and Brookes, P.: Cell-mediated mutagenesis in cultured Chinese hamster cells by polycyclic hydrocarbons: mutagenicity and DNA reaction related to carcinogenicity in a series of compounds. Int. J. Cancer, 23, 691, 1979.

9. Newbold, R.F., Brookes, P., and Harvey, R.G.: A quantitative comparison of the mutagenicity of carcinogenic polycyclic hydrocarbon derivatives in cultured mammalian cells. Int. J. Cancer, 24, 203, 1979.

10. Markov, G.G. and Ivanov, I.G.: Hydroxylapatite column chromatography in procedures for isolation of purified DNA. Anal. Biochem., 59, 555, 1974.

11. Harris, C.C., Frank, A.L., vanHaafter, C., Kaufman, D.G., Connor, R., Jackson, F., Barrett, L.A., McDowell, E.M., and Trump. B.F.: Binding of [^{3}H] benzo(a)pyrene to DNA in cultured human bronchus. Cancer Res., 36, 1011, 1976.

12. Jernström, B., Vadi, H. and Orrenius, S.: Formation of DNA-binding products from isolated benzo(a)pyrene metabolites in rat liver nuclei. Chem.-Biol. Interact., 20, 311, 1978.

13. Beland, F.A., Dooley, K.L., and Casciano, D.A.: Rapid isolation of carcinogen-bound DNA and RNA by hydroxyapatite. J. Chromatog., 174, 177, 1979.

14. Jeffrey, A.M., Weinstein, I.B., Jennette, K.W., Grzeskowiak, K. and Nakanishi, K.: Structures of benzo(a)pyrene-nucleic acid adducts formed in human and bovine bronchial explants. Nature, 269, 348, 1977.

15. Adriaenssens, P.I., Bixler, C.J., and Anderson, M.W.: Isolation and quantitation of DNA-bound benzo(a)pyrene metabolites: comparison of hydroxylapatite and precipitation procedures. Anal. Biochem., 123, 162, 1982.

16. Garrett, N.E., Campbell, J.A., Stack, H.F., Waters, M.D., and Lewtas, J.: The utilization of the rabbit alveolar macrophage and Chinese hamster ovary cell for evaluation of the toxicity of particulate materials. I: Model compounds and metal-coated fly-ash. Environ. Res., 24, 345, 1981.

17. Garrett, N.E., Campbell, J.A., Stack, H.F., and Jackson, M.A.: Cellular toxicity in Chinese hamster ovary cells. II: A statistical appraisal of sensitivity with the rabbit alveolar macrophage, Syrian hamster embryo, BALB 3T3 mouse, and human neonatal fibroblast cell systems. Environ. Res., (in press).

18. Howard, P.C. and Beland, F.A.: Xanthine oxidase catalyzed binding of 1-nitropyrene to DNA. Biochem. Biophys. Res. Commun., 104, 727, 1982.

19. Schneider W.C.: Determination of nucleic acids in tissues by pentose analysis. In: Methods in Enzymology Vol. 3, Eds. S.P. Colowick and N.O. Kaplan, Academic Press, New York, 1957, p. 680.

20. Ceriotti G.: Determination of nucleic acids in animal tissues. J. Biol. Chem., 214, 59, 1955.

21. Lowry, O.H., Rosebrough, N.G., Farr, A.L., and Randall, R.J.: Protein measurement with the Folin reagent. J. Biol. Chem., 193, 265, 1951.

22. Loring, H.S.: Hydrolysis of nucleic acids and procedures for the direct estimation of purine and pyrimidine fractions by absorption spectrophotometry. In: The Nucleic Acids, Vol. 1, Eds. E. Chargaff and J.N. Davidson. Academic Press, New York, 1955, p. 191.

23. Hofmann, F., Bauer, C., Munder, P.H., Reutter, W. and Dicker, K.: Changes in biochemical properties of alveolar macrophages during activation *in vivo*. Biochem. Soc. Trans., 6, 1074, 1978.

24. Boroujerdi, M., Kung, H., Wilson, A.G.E., and Anderson, M.W.: Metabolism and DNA binding of benzo(a)pyrene *in vivo* in the rat. Cancer Res., 41, 951, 1981.

25. Reid, W.D., Glick, J.M., and Krishna, G.: Metabolism of foreign compounds by alveolar macrophages of rabbits. Biochem. Biophys. Res. Commun., 49, 626, 1972.